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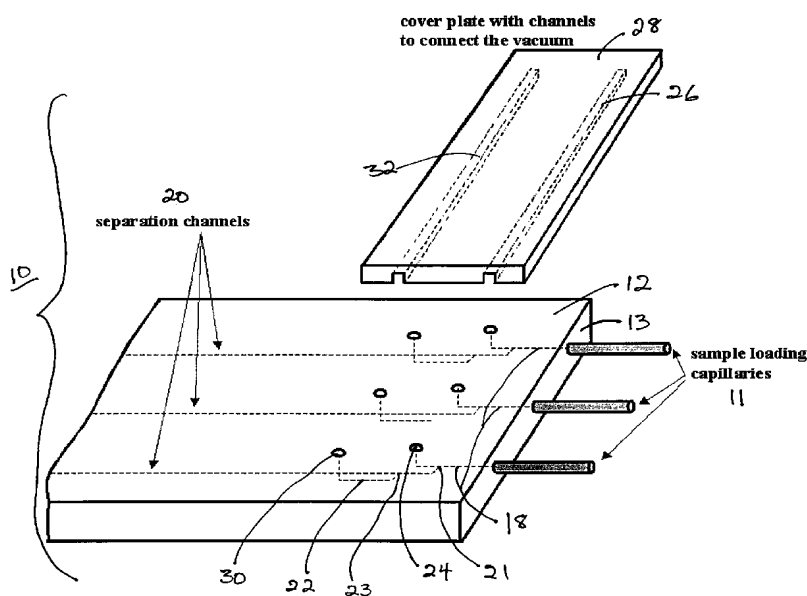
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(54) Title: PARALLEL SAMPLE LOADING AND INJECTION DEVICE FOR MULTICHANNEL MICROFLUIDIC DEVICES



(57) Abstract: A sample load and injection device (10) includes sample introduction capillaries (11) attached to a microfluidic device (12). Sample introduction capillaries (11) are connected to sample introduction channels (18). Sample introduction capillaries (18) are connected to separation channels (20) at connection points (21). At a defined distance along the separation channels (20), auxiliary channels (23) originate at connection points (24). The sample load and injection device includes cover plate (28) which has connecting channels (26, 32). Connecting channel (26) is associated with ends (24) of sample introduction channels (18). Connecting channel (32) is associated with ends (20).



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TITLE OF THE INVENTION

**Parallel Sample Loading and Injection Device for
Multichannel Microfluidic Devices**

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CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the priority of U.S. Provisional Application No. 60/176,183 filed January 18, 2000, and of PCT International Patent Application No. PCT/US00/09480 filed April 10, 2000, the whole of which are hereby incorporated by reference herein.

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STATEMENT REGARDING FEDERALLY SPONSORED

RESEARCH OR DEVELOPMENT

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Part of the work leading to this invention was carried out with United States Government support provided under a grant from the National Institutes of Health, Grant No. NIH (GM15847). Therefore, the U.S. Government has certain rights in this invention.

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BACKGROUND OF THE INVENTION

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Recent developments in microfabrication techniques have permitted the integration of microminiature tools for biochemical analysis within a tiny microfluidic device. Microfluidic devices have been successfully demonstrated as useful for a wide variety of separation techniques as well as for sample pretreatment and/or manipulation methods. Complete chemical processing systems, e.g., reaction chambers, separation capillaries and their associated electrode reservoirs, as well as certain types of detectors, can be consolidated on a

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microfluidic device or microchip. Such "labs-on-a-chip," in principle, permit effective utilization and manipulation of minute quantities of material. Systems have been developed that permit the efficient transfer of nanoliter quantities or other small quantities of a fluid sample from the spatially concentrated environment of a microfluidic device to "off-chip" analytical or collection devices without an increase in sample volume. (See, U.S. Patent No. 5,872,010, the whole of which is hereby incorporated by reference herein.) However, methods for moving small quantities of samples from a sample vial or holder onto a chip or directly to an analytical device without either evaporation or dilution are less well developed.

One important issue for the further development of microfluidic devices is that sample introduction into a device and subsequent injection of a sample aliquot into, e.g., a separation channel or reaction compartment on the device must be carried out in a reliable, accurate and convenient manner. Moreover, the method should not result in any sample "cross talk" and should be easy to automate. This is very difficult to accomplish when multiple channels on one microfluidic device are used.

BRIEF SUMMARY OF THE INVENTION

The invention is directed to a universal interface and sample load and injection device, and to method for its use, for the parallel introduction and simultaneous or subsequent injection of liquid samples into channels of a multichannel microfluidic device. The sample load and injection device can be integral with a multichannel

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microfluidic device or can be a separately attachable entity. The sample load and injection device of the invention comprises a sample introduction capillary (and, preferably, multiple sample introduction capillaries), each said introduction capillary having an inlet end and an outlet end, the inlet end of each said individual capillary being external to said sample load and injection device and capable of being placed in alignment with one of multiple samples in a sample holder, e.g., one well in a 96-well microtiter plate, and the outlet end of each said introduction capillary opening to an introduction channel in said sample load and injection device, each introduction channel an end within the device. The sample load device further comprises a sample channel originating at each said introduction channel, an auxiliary channel originating at each said sample channel and ending at an auxiliary channel end within said sample load and injection device, a connecting channel connecting said introduction channel ends and a connecting channel connecting said auxiliary channel ends. The connecting channel for said introduction channel ends and the connecting channel for said auxiliary channel ends each terminates at a surface of said sample load device, and each said connecting channel is configured for connection to a source of positive or negative pressure or to a source for applying an electric field across any channel segment. The sample channels and the auxiliary channels are preferably smaller in internal diameter than the introduction channels.

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In other embodiments, the connecting channels reside in a cover plate that is separable from the sample load and injection device body and at least one of the sample channels is configured for separation of a loaded sample. Separation can be of any kind, e.g., chromatographic (LC, CEC) or electrophoretic (CZE, IEF, MEKCC). Channels configured for separation can be equipped, e.g., for absorbance (UV, Vis), fluorescence, conductivity, electrochemical detection or for sample collection or transfer to external devices such as MALDI/TOF. Furthermore, the sample load and injection device can include an electrospray interface chamber coupled to the outlet end of one of the sample introduction channels.

The sample load and injection device of the invention is suitable for moving small quantities of samples from, e.g., the wells of a 96-well microtiter plate (or any other sample plate size), onto a microfluidic device or directly to an analytical device, without either evaporation or dilution, and for, e.g., sample preconcentration, entrapment or preseparation prior to further manipulation on the microdevice. The sample introduction (or loading) capillaries can be packed with any suitable material (e.g., chromatographic beads or polymeric monolith, affinity material, immobilized enzyme material, etc.) or coated with any kind of coating so that, e.g., most of the components of the sample (or only selected components) can be trapped or digested inside the loading capillaries. After trapping, the sample constituents can be eluted into the channels inside the microdevice with the use of a suitable eluent. Selective stepwise or gradient elution

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can be applied to elute only certain sample components at a time. Individual sample loading capillaries can contain different packing materials and have different internal volumes so that different sample volumes can be handled in individual channels of the accompanying microfluidic device. Additionally, the sizes of the channels inside the microfluidic device can be optimized so that different flow rates are generated upon application of a pressure or electric field. This flexibility is useful to increase the dynamic range of an analysis carried out using the device of the invention. If the packing inside individual sample loading capillaries is not functionalized, it can, at least, serve as a particle filter to prevent clogging of channels internal to the device. The sample loading capillaries can be designed either as integral parts of the device (e.g., pulled from the device) or as separately attachable entities. They can be reusable or disposable.

A sample loading and injection device can be made of any etchable or moldable material. Preferably, the material is a transparent plastic or glass (e.g., silicon). Various metals are useful for certain applications.

The samples may be transferred using the sample loading and injection device of the invention for electrospray-mass spectrometry analysis (ESI/MS), for atmospheric pressure-chemical ionization mass spectrometry analysis (APCI/MS), for matrix assisted laser desorption ionization mass spectrometry (MALDI/MS), for nuclear magnetic resonance analysis (NMR), for pneumatically or ultrasonically assisted spray sample

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handling, for transfer to an off-chip detection system,
such as electrochemistry, conductivity or laser induced
fluorescence, or for collection of specific fractions,
e.g., in collection capillaries or on collection
5 membranes.

The channels of any microfluidic device used with
the sample load mechanism of the invention may be arrayed
in any format that allows for sequential or simultaneous
processing of liquid samples, as desired.

10 In any embodiment, each channel may include
electrical contacts, so that an electric circuit path can
be established along the channel. For example, one
electrical contact can be on the entrance side of a
channel and another electrical contact can be on the exit
15 side. In an alternative arrangement, an electric circuit
can be completed by an external contact, beyond the exit
end of the channel. For example, if the exit port of a
channel is used as an electrospray source for a mass
spectrometer, the mass spectrometer sampling orifice can
20 serve as the counter electrode. Samples can be
transferred off chip for subsequent analysis by switching
the electric current sequentially to each channel on the
chip.

Samples can be introduced into a channel on a
25 microfluidic device by the sample loading and injection
device of the invention by a variety of methods, e.g., by
pressure, electrokinetic injection, or other technique,
and an electrical current and/or pressure drop can then
be applied to cause the sample components to migrate
30 along the channel. The channels may function only for
fluid transfer, e.g., to a mass spectrometer, or the

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channels can serve as environments for various types of sample manipulations, e.g., for micropreparative or analytical operations, such as capillary electrophoresis (CE), chromatography or the polymerase chain reaction (PCR), or for carrying out any type of sample chemistry. The channels may be filled with membrane or packing material to effectuate preconcentration or enrichment of samples or for other treatment steps, such as desalting. Furthermore, other modification of sample components, e.g., by enzymes that are covalently bound to the walls of a channel or are free in a channel, are possible. Packing material may be bound to the walls of the channels or may include other components, such as magnetic particles, so that when a magnetic field is applied, the magnetic particles retain the packing material in place. The magnetic particles can also be used for efficient mixing of fluids inside the channels, using an external magnetic field. A micromachined filter or other stationary structure may also be employed to hold packing material in place. Alternatively, stationary structures can be micromachined, cast or otherwise formed in the surface of a channel to provide a high surface area which can substitute for packing material.

A sample can be introduced into a channel in a short starting zone or can fill the whole channel completely. Filling only a small part of the channel with the sample is preferable when an on-chip separation of sample components is to be carried out, such as electrophoresis or chromatography. Filling the whole channel with the sample may be advantageous in cases when off-chip

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analysis requires extended sample outflow, such as sample introduction/electrospray ionization for structure analysis by mass spectrometry.

5 In many cases a liquid flow may be required to transport the analytes in a sample into a specific channel, or along the length of the channel, or out of the channel via an exit port. Therefore, to assist in the required fluid transfer, a pumping device may be incorporated into or associated with the microscale
10 device of the invention. For example, a heating element can be used to cause thermal expansion, which will effectuate sample liquid movement, or a heating element can be used to generate a micro bubble, the expansion of which causes the sample to travel in the channel. Other
15 options may include pumping by the pressure of a gas or gases generated by on-chip electrolysis. Flow can be also generated by application of a pressure drop along a channel or by electroosmosis inside a channel.

20 As samples move to the end of a channel, they can be subjected to detection or analysis at a site external to the microscale device of the invention by a variety of techniques, including mass spectroscopy, nuclear magnetic resonance, laser induced fluorescence, ultraviolet detection, electrochemical detection, or the like. The
25 end of each channel may include a tip configured to facilitate transfer of the sample volume.

Different sized channels may be employed on the same accompanying microfluidic device. For example, larger channels may be used for cleanup operations, and smaller
30 channels may be used for processing operations. Moreover, other operations can be performed in other

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regions of the device, such as chemical processing, separation, isolation or detection of a sample or a component of the sample, prior to sample loading in a channel. Thus, it is possible to carry out sample chemistries or to conduct micropreparative and analytical operations on both a starting sample and its separated components within the device of the invention, prior to transfer of the sample or its components off chip for further analysis or collection. Additionally, detection of a sample may be carried out on the microfluidic device itself, e.g., by a fiber optic detection system, which can provide complementary control information for off-chip analysis and detection, or by any other suitable detector such as laser induced fluorescence, conductivity and/or electrochemical detector.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING

Fig. 1a is a plan view of a sample load and injection device according to the invention;

Fig. 1b is a section through the sample load and injection device of Fig. 1a;

Fig. 2 is an exploded perspective view of a sample load and injection device according to the invention; and

Fig. 3 is an exploded perspective view of another embodiment of a sample load and injection device according to the invention.

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DESCRIPTION OF THE PREFERRED EMBODIMENT OF THE INVENTION

The parallel sample load and injection device of the invention allows for a simple automated way of transferring samples from a standard, e.g., 96-well, microtiter plate into a microfluidic device and for the simultaneous or subsequent injection of samples into multiple channels on the device.

Referring to Figs. 1a - 3, a sample load and injection device (10) includes parallel sample introduction capillaries (11) that are attached to a microfluidic device (12) into which the sample load and injection device of the invention is integrated. The spacing between these capillaries is compatible with the spacing of the wells in standard microtiter plates (14). As indicated in Fig. 3, individual sample introduction capillaries (11) may contain packing material (15) for sample pretreatment.

Sample solutions in individual wells (16) are transferred through introduction capillaries (11) into introduction channels (18) in the sample load and injection device. Each of the sample introduction channels (18) is connected to the appropriate sample separation channel (20), at connection point (21). At a defined distance along the separation channel, an auxiliary channel (22), which aids in sample injection as will be described below, originates at connection point (23). The distance between these two connection points determines the length of the sample injection "plug." All sample introduction channels are connected through introduction channel ends (24) to a connecting channel (26) made in associated cover plate (28), which

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is attachable to device (12). Channel (26) is used for applying negative pressure (a vacuum) simultaneously to all sample introduction channels (18). A similar connection is made among associated auxiliary channel ends (30), using connecting channel (32). Sample separation channels (20) and auxiliary channels (22), (generally 200-1000 μL in internal diameter and preferably approximately 500 μL) are preferably smaller in internal diameter than introduction channels (18), which are generally 20-100 μL (and preferably 50 μL) in internal diameter.

In one embodiment, sample loading and injection can be carried out in the following manner: the channels of the microfluidic device are first flushed with background electrolyte, and then the device is positioned in front of a microtiter plate so that the sample introduction capillaries (11) are immersed into individual sample wells. A vacuum is applied to connection channel (26), which connects introduction channel ends (24). The different samples simultaneously fill the sample introduction channels (18), but due to hydrodynamic resistance, do not enter the smaller sample separation channels (20). This is the loading step. Subsequently, in a similar manner, connection channel (32) is used for application of a vacuum to all auxiliary channels so that the samples are pulled through a portion of the separation channels into the auxiliary channels; this is the injection step. After this step, the microtiter plate is replaced by a reservoir containing background electrolyte. A vacuum is then again applied to the sample introduction channels (18), via connecting channel (26),

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so that the content of these channels is exchanged for background electrolyte. The sample injection "plugs," i.e., those portions of the samples that were introduced into the sections of the separation channels between connection points (21) and (23), remain unchanged since the high hydrodynamic resistance of the channels (20) and (22) minimizes sample flow. (Sample also remains in auxiliary channels (22).) Subsequently, e.g., in order to separate the components of the sample in the sample plugs, an electrical potential is applied across the separation channels.

The method of the invention can also be carried out in a manner that combines the loading and injection steps. In this procedure, a vacuum is applied first to connection channel (32) so that the individual samples are pulled directly through a portion of loading channels (18) and separation channels (20) and into auxiliary channels (22). For practice of this method of the invention, the separation and auxiliary channels need not be smaller in internal diameter than the loading channels. Simultaneous loading and injection of samples is a faster procedure. Sequential loading and injection steps are useful when it is desirable to carry out, e.g., sample preconcentration, entrapment or preseparation in the loading capillaries prior to sample injection.

The disclosed sample introduction and injection procedure will be useful for any device designed for handling and analysis of small amounts of samples, especially in genomics and proteomics. The parallel sample load and injection device and method of the invention allow for a simple automated way for the

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transfer of samples from a standard microtiter plate into a microfluidic device and simultaneous injection into multiple channels on the device. The method of the invention has been described for hydrodynamic sample
5 injection followed by electrophoretic separation. However, the method of the invention can be also used for electrokinetic injection and for any other kind of separation or manipulation technique.

The sample load mechanism of the invention permits
10 more efficient use of powerful analytical devices, such as the mass spectrometer, than is currently possible. In addition, the system of the invention can be manufactured as a disposable device that is suitable for cost effective automation of the analysis of a large number of
15 samples. Using this micromachined approach, high throughput analysis by mass spectrometry would be possible. In addition, handling of small volumes and quantities of samples would be facilitated, and consumption of valuable samples and reagents would be
20 reduced. Applications include any laboratory analysis methods, especially where high throughput and minimization of cross-contamination are desirable, such as screening and diagnostic methods, and such other analytic methods as pharmacokinetics where fresh columns
25 are required for each run.

While the present invention has been described in conjunction with a preferred embodiment, one of ordinary skill, after reading the foregoing specification, will be able to effect various changes, substitutions of
30 equivalents, and other alterations to the compositions and methods set forth herein. It is therefore intended

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that the protection granted by Letters Patent hereon be limited only by the definitions contained in the appended claims and equivalents thereof.

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CLAIMS

What is claimed is:

1. A sample load and injection device for the parallel
5 transfer of liquid samples into channels of a
multichannel microfluidic device, said sample load and
injection device comprising:

multiple sample introduction capillaries, each said
introduction capillary having an inlet end and an outlet
10 end, the inlet end of each said individual capillary
being external to said sample load and injection device
and capable of being placed in alignment with one of
multiple samples in a sample holder, and the outlet end
of each said introduction capillary opening to an
15 introduction channel in said sample load and injection
device, said introduction channel having an introduction
channel end in said sample load and injection device;

a sample channel originating at each said
introduction channel;

20 an auxiliary channel originating at each said sample
channel and ending at an auxiliary channel end in said
sample load and injection device;

a connecting channel connecting said introduction
channel ends; and

25 a connecting channel connecting said auxiliary
channel ends, said connecting channel for said
introduction channel ends and said connecting channel for
said auxiliary channel ends each terminating at one end
at a surface of said sample load and injection device,
30 wherein each said connecting channel is configured for
connection to a source of positive or negative pressure

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or to a source for applying an electric field across any channel segment.

2. The sample load and injection device of claim 1,
5 wherein said sample channels and said auxiliary channels are smaller in internal diameter than are said introduction channels.

3. The sample load and injection device of claim 1,
10 wherein said connecting channels are resident in a cover plate that is separable from the main body of said sample load and injection device.

4. The sample load and injection device of claim 1,
15 wherein said one or more of said sample channels is configured for separation of a loaded sample.

5. The sample load and injection device of claim 1,
20 wherein said sample load and injection device is integral with a multichannel microfluidic device.

6. The sample load and injection device of claim 1,
25 wherein said sample load and injection device is separable from but attachable to a multichannel microfluidic device.

7. A method for the parallel injection of samples into separate channel systems within a microfluidic device, said method comprising the steps of:

30 providing the sample load and injection device of claim 1;

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positioning said sample load and injection device adjacent a sample holder having multiple individual samples so that individual sample introduction capillaries of said device are immersed into individual samples in said sample holder;

5 applying a vacuum to said connecting channel connecting said auxiliary channel ends, wherein said individual samples are pulled through said introduction channels and a portion of each of said separation channels and into said auxiliary channels; and

10 replacing said individual samples in said sample holder by a reservoir containing a background electrolyte and applying a vacuum to said connecting channel connecting said introduction channel ends, whereby the portions of said samples remaining in said introduction capillaries and said introduction channels are replaced by said background electrolyte.

8. The method of claim 7, wherein, prior to the step of applying a vacuum to said connecting channel connecting said auxiliary channel ends, said method further comprises the step of applying a vacuum to said connecting channel connecting said introduction channel ends, wherein said individual samples simultaneously fill said individual sample introduction capillaries and associated sample introduction channels.

9. The method of claim 7 or claim 8, wherein, prior to said positioning step, said method further comprises the step of flushing said channels of said device with background electrolyte.

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10. The method of claim 7 or claim 8, wherein said sample introduction capillaries comprise a coating or a packing material and said samples are exposed to said coating or to said packing material during passage through said introduction capillaries.

11. The method of claim 7 or claim 8, wherein samples in said introduction capillaries are subjected to an isoelectrofocusing step.

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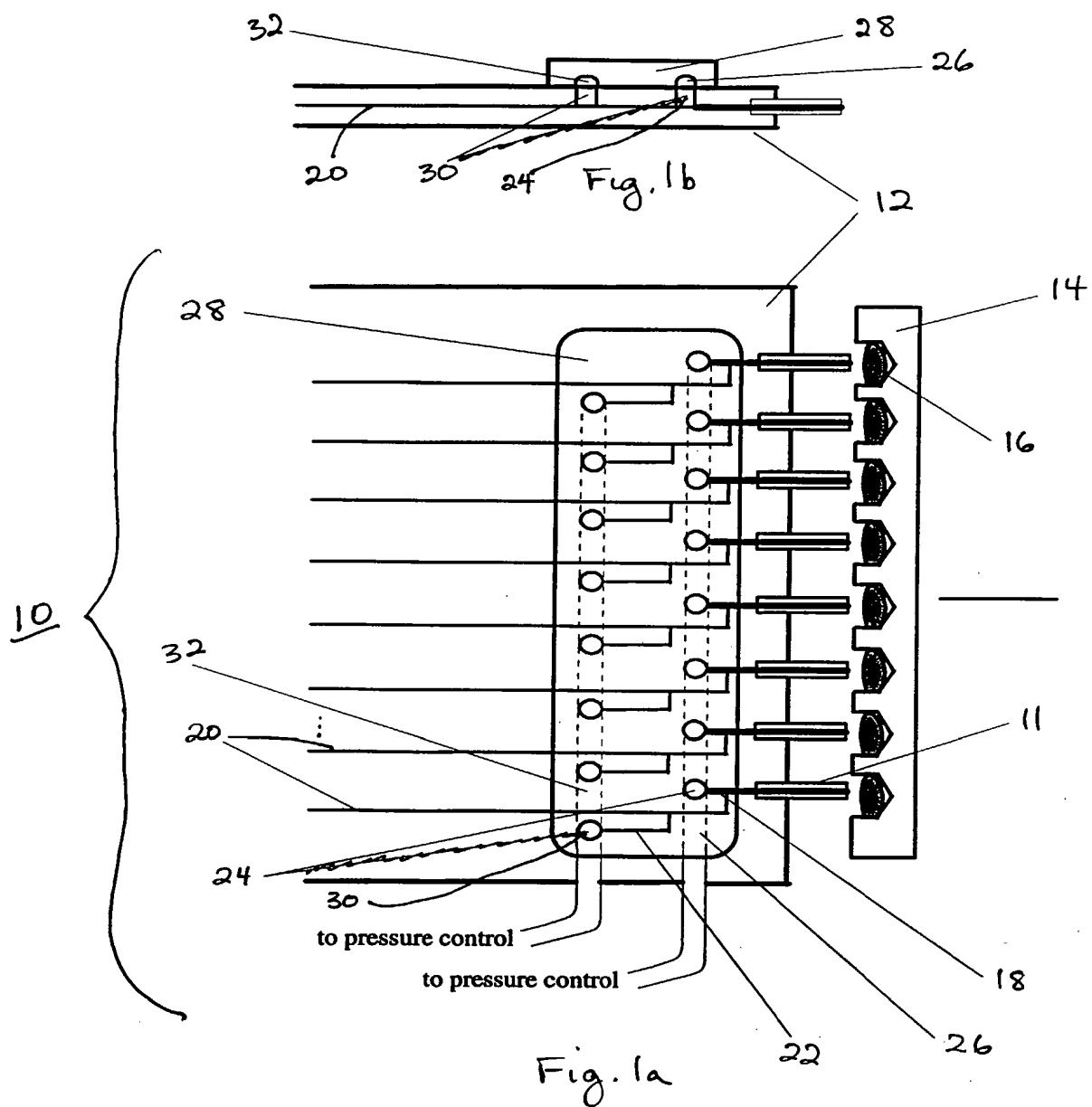


Fig. 1 Scheme of parallel sample injection

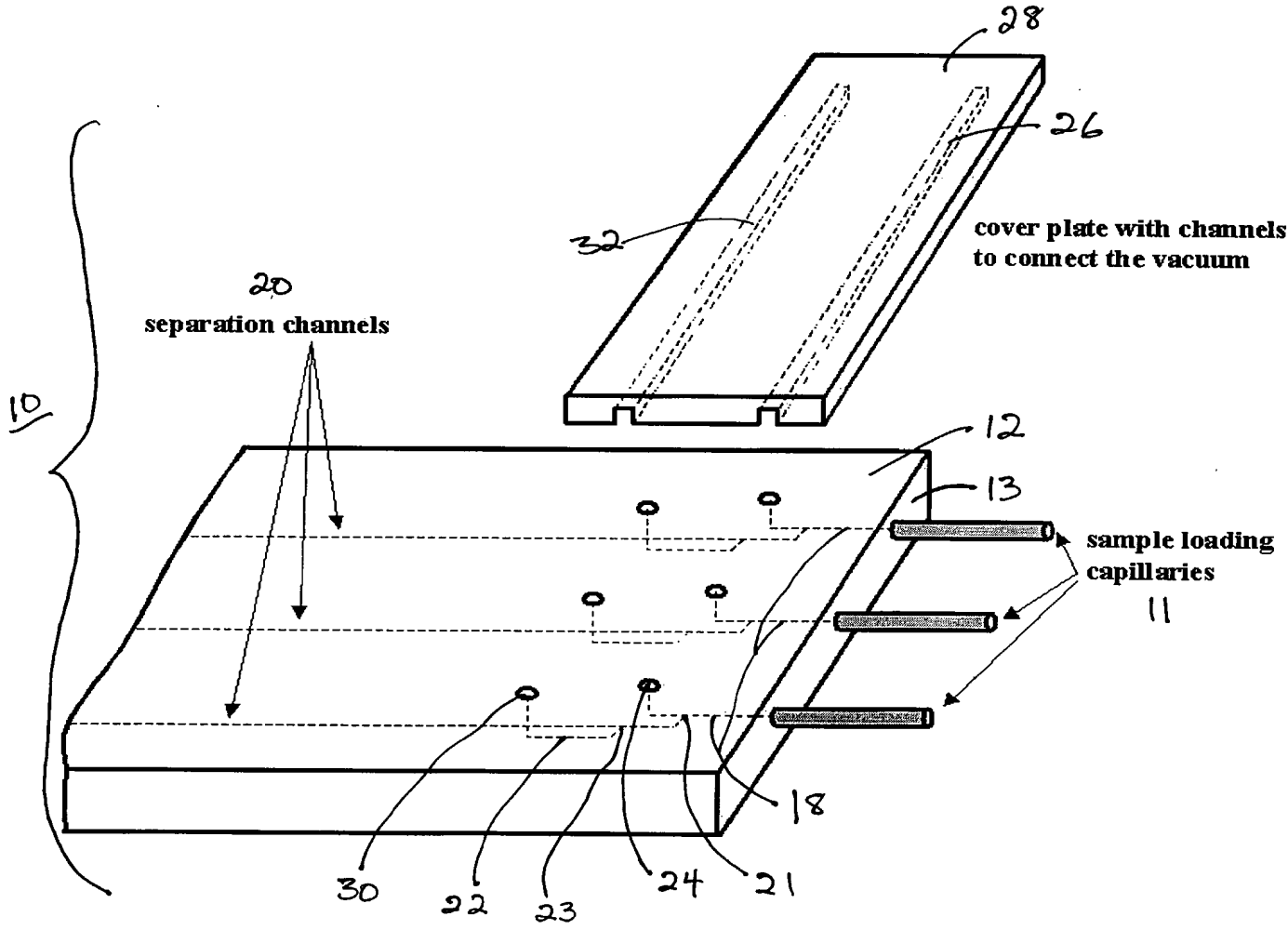


Fig. 2

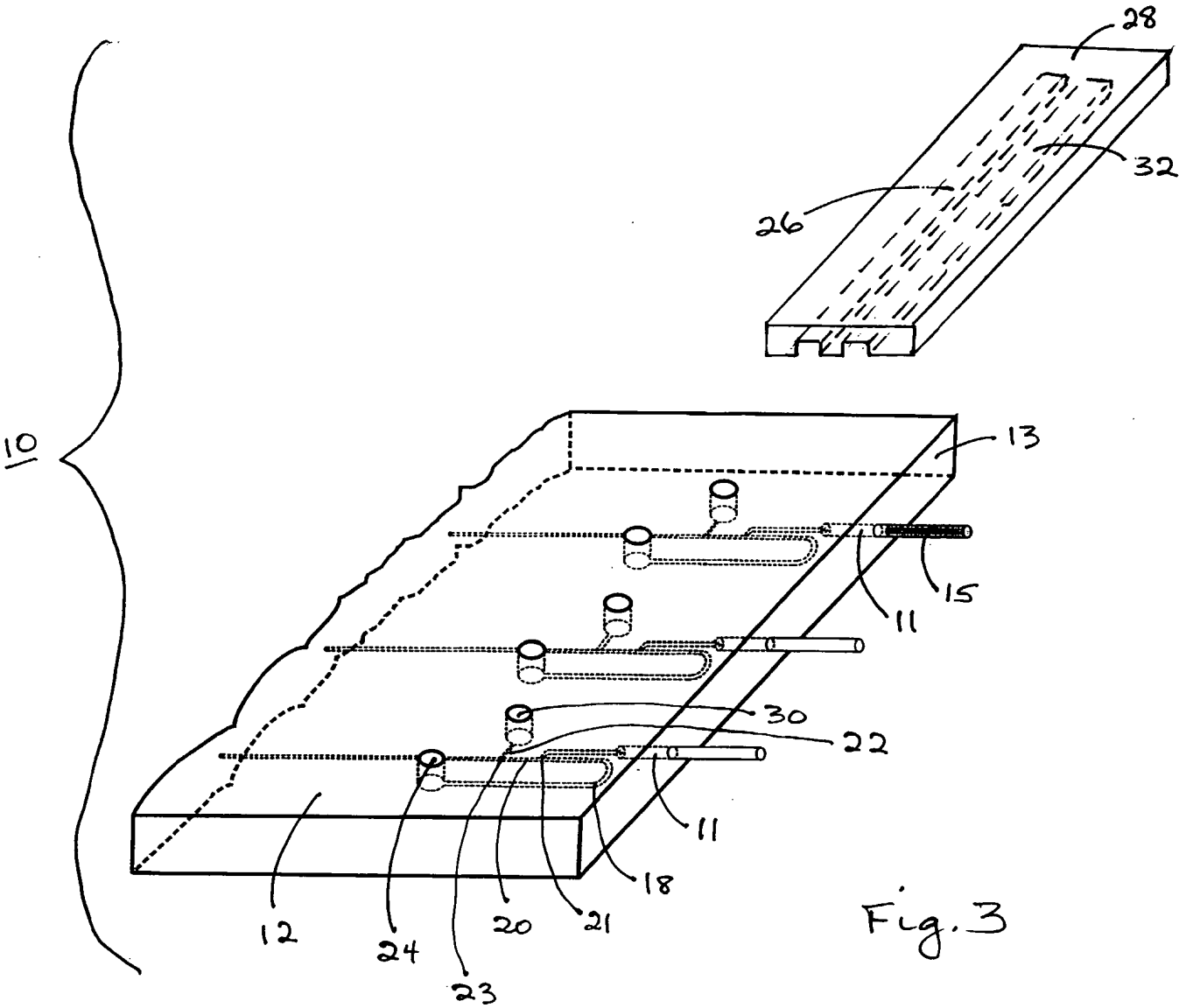


Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/01705

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G01N 1/10, 27/26, 27/344

US CL :204/450.451,453,600,601,604; 422/100; 436/180

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 204/450.451,453,600,601,604; 422/100; 436/180

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,872,010 A (KARGER et al) 16 February 1999	1-11
A	US 5,885,430 A (KERMAN et al) 23 March 1999	1-11
A,P	US 6,086,825 (SUNDBERG et al) 11 July 2000	1-11
A,P	US 6,110,332 A (SWIERKOWSKI) 29 August 2000	1-11
A,P	US 6,149,787 A (CHOW et al) 21 November 2000	1-11
A,P	US 6,165,417 A (SWIERKOWSKI) 26 Decenber 2000	1-11
A,E	US 6,207,031 B1 (ADOURIAN et al) 27 March 2001	1-11



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

10 APRIL 2001

Date of mailing of the international search report

08 MAY 2001

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/01705

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

USPAT,JPOABS,EPOABS,DERWENT,CAPLUS

search terms: microchip, microchannel,microconduit, microfluidic, microfabricat?, microanal?, microlab?, microscale, nanoscale, mesoscale, lab? (3w) (chip or microchip), sample, load?, inject?, introduc?